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(54) Title: CYTOKINE DESIGNATED LERK-8

(57) Abstract

The invention is directed to a protein designated Lerk-8, DNA encoding the Lerk-8, and host cells transformed with Lerk-8 DNA. Antibodies that are immunoreactive with Lerk-8 are also provided. The Lerk-8 protein binds to the cell surface receptors known as elk and hek.

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TITLE
CYTOKINE DESIGNATED LERK-8

BACKGROUND OF THE INVENTION

Proteins known as the receptor tyrosine kinases have an intrinsic kinase activity that is activated upon ligand binding. This class of proteins is characterized by conserved structural motifs within the catalytic domains (Hanks et al., *Science*, 242:42, 1988) and can be subdivided into families based on structural features of the regions N-terminal to the catalytic domain.

The *eph* family of receptors, named after the first member isolated (Hirai et al., *Science* 238:1717, 1987) is the largest subfamily of receptor tyrosine kinases. Among the members of this family are chicken cek4 (Sajjadi et al. *New Biol.* 3:769, 1991) and cek5 (Pasquale, E.B., *Cell Regulation* 2:523, 1991); murine mek4 (Sajjadi et al., *supra*), bsk (Zhou et al., *J. Neurosci. Res.*, 37:129, 1994), nuk (Henkemeyer et al., *Oncogene* 9:1001, 1994), and sek (Gilardi-Hebenstreit et al., *Oncogene* 7:2499, 1992); rat elk (Letwin et al., *Oncogene* 3:621, 1988; Lhotak et al., *Mol. Cell. Biol.* 11:2496, 1991), eek (Chan et al., *Oncogene* 6:1057, 1991), ehk-1 and ehk-2 (Maisonpierre et al., *Oncogene* 8:3277, 1993): and human hek (Boyd et al., *J. Biol. Chem.*, 267:3262, 1992; Wicks et al., *PNAS USA*, 89:1611, 1992), hek2 (Bohme et al., *Oncogene* 8:2857, 1993), eck (Lindberg et al. *Mol. Cell. Biol.* 10:6316, 1990), and erk (Chan et al., *supra*).

The proteins of this subfamily are related not only in their cytoplasmic domains, but also in their extracellular domains, which are 41 to 68% identical. Interestingly, the tissue distributions of these various receptors are diverse. Because many eph-related receptor tyrosine kinases are primarily expressed in the brain, it has been postulated that these receptors and their ligands may be involved in the growth, differentiation, and survival of neurons.

Those ligands that have been identified for the receptor tyrosine kinases are a diverse group of proteins that affect the growth, differentiation, and survival of cells expressing the receptors. Certain ligands have been found to bind to more than one receptor of the eph family. Examples are the ligands for hek and elk that are described below.

Identification of additional ligands for hek and elk that may exist would prove useful in investigating the nature of cellular processes regulated by signaling through these receptors. If enhancement or inhibition of a particular biological signal mediated through

these receptors is desired, it is advantageous to identify each of the proteins that may play a role in transduction of such signals. Further, it is known that certain proteins can bind to receptors without initiating signal transduction, including interleukin-1 receptor antagonist protein (Eisenberg et al., *Nature* 343:341, 1990; Hannum et al., *Nature* 343:336, 1990; and Carter et al., *Nature* 344:633, 1990). Identification of additional proteins that bind hek or elk is also desirable in order to determine whether any of such proteins functions as an antagonist.

SUMMARY OF THE INVENTION

The present invention is directed to a novel cytokine designated Lerk-8. Lerk-8 binds to the cell surface receptors known as hek and elk, which are members of the above-described *eph/elk* family of receptor tyrosine kinases.

Purified Lerk-8 proteins are provided herein, along with isolated DNAs encoding Lerk-8, expression vectors comprising the Lerk-8 DNA, and host cells transformed with the expression vectors. Processes for producing Lerk-8 include culturing such transformed host cells under conditions that promote expression of Lerk-8 polypeptides, and recovering the Lerk-8. The invention also encompasses antibodies that are directed against Lerk-8.

DETAILED DESCRIPTION OF THE INVENTION

A novel cytokine designated Lerk-8 is provided herein. This cytokine binds to the receptor tyrosine kinases known as elk and hek.

The present invention encompasses DNA encoding Lerk-8, expression vectors comprising the Lerk-8 DNA, and host cells transformed with the expression vectors. A method for producing Lerk-8 polypeptides comprises culturing the transformed host cells under conditions conducive to expression of Lerk-8, and recovering the expressed Lerk-8. Purified Lerk-8 polypeptides in both soluble and membrane-bound form are disclosed.

Lerk-8 polypeptides or immunogenic fragments thereof may be employed as immunogens to generate antibodies that are immunoreactive therewith. In one embodiment of the invention, the antibodies are monoclonal antibodies.

A cDNA encoding human Lerk-8 was isolated as described in example 1. The nucleotide sequence of this Lerk-8 cDNA is presented in SEQ ID NO:1, and the amino acid sequence encoded thereby is presented in SEQ ID NO:2. This Lerk-8 protein comprises an N-terminal signal peptide (amino acids -27 to -1), an extracellular domain (amino acids 1 to 197), a transmembrane region (amino acids 198 to 224), and a cytoplasmic domain (amino acids 225 to 313).

The calculated molecular weight of the mature human Lerk-8 protein (amino acids 1 to 313 of SEQ ID NO:2) is about 33 kilodaltons, and the isoelectric point (pI) is 8.46. One

embodiment of the present invention thus is directed to a purified human Lerk-8 protein characterized by a calculated molecular weight of about 33 kilodaltons and a pI of 8.46, wherein the N-terminal amino acid sequence of a mature form of the protein is Leu-Ser-Leu-Glu-Pro-Val-Tyr-Trp-Asn-Ser-Ala-Asn- (amino acids 1-12 of SEQ ID NO:2). The calculated molecular weight is based on the molecular weight of a protein having the specified amino acid sequence, exclusive of any glycosylation. The skilled artisan will recognize that glycosylated forms of the protein will have a higher molecular weight.

Lerk-8 fragments, e.g., fragments that retain the ability to bind hek or elk, are provided as well. Examples of such fragments are soluble Lerk-8 polypeptides.

The present invention provides both cell membrane-bound and soluble (secreted) forms of Lerk-8. Soluble Lerk-8 polypeptides include the receptor-binding domain of a Lerk-8, but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. In one embodiment, a soluble Lerk-8 comprises the entire extracellular domain (e.g., amino acids 1 to 197 of the human Lerk-8 of SEQ ID NO:2). In another alternative, the soluble polypeptide is a fragment of the Lerk-8 extracellular domain that retains the ability to bind elk or hek. The portion of the extracellular domain believed to be most important for receptor binding includes amino acids 1 to 142 of SEQ ID NO:2. The remainder of the extracellular domain (amino acids 143 to 197) constitutes a spacer region.

Examples of soluble human Lerk-8 polypeptides include, but are not limited to, polypeptides truncated at the C-terminus so that the C-terminal amino acid is any of the residues between or including the residues at positions 142 and 197 of SEQ ID NO:2. In other words, such soluble Lerk-8 polypeptides comprise amino acids 1 to y of SEQ ID NO:2, wherein y is any integer from 142 through 197.

Soluble Lerk-8 may be identified (and distinguished from its non-soluble membrane-bound counterparts) by separating intact cells expressing a Lerk-8 polypeptide from the culture medium, e.g., by centrifugation, and assaying the medium (supernatant) for the presence of the desired protein. The presence of Lerk-8 in the medium indicates that the protein was secreted from the cells and thus is a soluble form of the desired protein.

Soluble forms of Lerk-8 possess certain advantages over the membrane-bound form of the protein. Purification of the protein from recombinant host cells is facilitated, since the soluble proteins are secreted from the cells. Further, soluble proteins are generally more suitable for certain applications, e.g., for intravenous administration.

When initially expressed within a host cell, soluble Lerk-8 polypeptides advantageously comprise the native signal peptide or one of the heterologous leader or signal peptides described below that is functional within the host cells employed. Isolated DNA sequences encoding soluble Lerk-8 proteins are encompassed by the present invention.

Truncated Lerk-8, including soluble polypeptides, may be prepared by any of a number of conventional techniques. A DNA sequence encoding a truncated Lerk-8 may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. Oligonucleotides that reconstruct the 5' or 3'-terminus of a DNA fragment to a desired point may be utilized. Linkers containing restriction endonuclease cleavage site(s) may be employed to insert the desired DNA fragment into an expression vector. The well known polymerase chain reaction (PCR) procedure also may be employed to amplify a DNA fragment encoding a particular protein fragment. Primers that define the desired termini of the DNA fragment are employed in the PCR. As a further alternative, known mutagenesis techniques may be employed to insert a stop codon at a desired point, e.g., immediately downstream of the codon for the last amino acid of the receptor-binding domain.

An expressed sequence tag (EST) contains regions of identity with SEQ ID NO:1 (see example 1). The computer databank record for this EST (accession no. H10006) presents a DNA sequence 454 nucleotides in length. When the EST H10006 sequence is aligned with SEQ ID NO:1, regions of identity are found between nucleotides 663 and 1118 of SEQ ID NO:1. Certain of the nucleotides in EST H10006 are unidentified (i.e., are designated "N" in the databank record because their identity was unknown). The EST sequence contains inserted nucleotides not found in the corresponding positions in SEQ ID NO:1, as well as deletions and mismatches when compared to SEQ ID NO:1.

No reading frame is identified in the databank file for the EST, and the sequence lacks an initiation codon. Further, the above-mentioned insertions and deletions would cause shifts in the reading frame, compared to the reading frame of the Lerk-8 sequence of SEQ ID NO:1. However, even if a reading frame had been elucidated and identified, and adjustments made for the inserted, deleted, and unidentified nucleotides, a translate of EST H10006 would lack one of the four cysteine residues that are conserved in the other Lerk proteins (described below), and would lack other conserved residues as well. The four conserved cysteines are believed to be important for the property of binding to elk and hek.

Other proteins that bind to both hek and elk have been discovered, and are designated Lerk-1 through Lerk-7 (ligands of the eph-related kinases). Lerks 2 and 5 are type 1 transmembrane proteins (as is Lerk-8), while Lerks 1, 3, 4, 6, and 7 are anchored to the cell membrane by GPI linkage. The percent identity of the amino acid sequences of these six proteins ranges from about 24 to 59%, and the proteins each have four conserved cysteine residues.

Holzman et al. (*Mol. Cell. Biol.* 10:5830, 1990) reported the cloning of cDNA for a protein called B61. The ability of B61 to bind to elk and to hek was discovered

subsequently, and the B61 protein was given the alternative designation Lerk-1 (Beckmann et al., *EMBO J. 13*:3757, 1994). B61 has also been reported to be a ligand for the above-described receptor tyrosine kinase known as eck (Bartley et al., *Nature* 368:558, 1994).

Lerk-2, also known as elk ligand, is described in PCT application WO 94/11384. Lerk-3 and Lerk-4, also known as hek ligands, are both described in PCT application WO 95/06065, Lerk-5 is described in WO 96/01839, Lerk-6 in WO 96/10911, and Lerk-7 in WO 96/17925.

The percent identity of the human Lerk-8 amino acid sequence of SEQ ID NO:2 with the full length amino acid sequence of various other proteins is as follows, wherein "h" represents human, "m" represents mouse, and "r" represents rat.:

h Lerk-1	25.14
h Lerk-2	40.80
r Lerk-2	39.69
m Lerk-2	40.00
h Lerk-3	24.88
h Lerk-4	25.41
h Lerk-5	41.23
m Lerk-5	42.07
m Lerk-6	26.18
h Lerk-7	24.88

As used herein, the term "Lerk-8" refers to a genus of polypeptides that are substantially homologous to the human Lerk-8 protein described in example 1. The polypeptides preferably comprise an amino acid sequence that is at least 80% identical, and more preferably at least 90% identical, to the amino acid sequence of SEQ ID NO:2, as further described below. The Lerk-8 polypeptides are capable of binding to the above-described receptors designated hek and elk. Certain uses of Lerk-8 flow from this ability to bind to elk or hek, as described in more detail below. Human Lerk-8 nucleic acids and proteins are within the scope of the present invention, as are Lerk-8 nucleic acids and proteins derived from other mammalian species that include but are not limited to murine, bovine, porcine, equine, or various primate species.

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in SEQ ID NO:1 and still encode a Lerk-8 protein having the amino acid sequence of SEQ ID NO:2. Such variant DNA sequences may result from silent mutations (e.g., occurring during PCR amplification), or may be the product of deliberate mutagenesis of a native sequence. The

present invention thus provides isolated DNA sequences selected from native Lerk-8 DNA sequences (e.g., cDNA comprising the nucleotide sequence presented in SEQ ID NO:1) and DNA that is degenerate as a result of the genetic code to a native Lerk-8 DNA sequence.

The Lerk-8 polypeptides provided herein include variants of native Lerk-8 polypeptides that retain a biological activity of a native Lerk-8. Such variants include polypeptides that are substantially homologous to native Lerk-8, but which have an amino acid sequence different from that of a native Lerk-8 because of one or more deletions, insertions or substitutions. Likewise, the Lerk-8-encoding DNAs of the present invention include variants that differ from a native Lerk-8 DNA sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active Lerk-8 polypeptide. The term "biologically active" as it refers to Lerk-8, indicates that the Lerk-8 is capable of binding to hek or to elk.

The variant DNA or amino acid sequences preferably are at least 80% identical to a native Lerk-8 sequence, most preferably at least 90% identical. The percent identity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Additional embodiments of variant amino acid sequences are those comprising conservative substitutions, meaning that one or more amino acid residues of a native Lerk-8 is replaced by a different residue, but that the conservatively substituted Lerk-8 polypeptide retains a desired biological activity of the native protein (e.g., the ability to bind elk or hek). Examples of conservative substitutions include substitution of residues that do not alter the secondary or tertiary structure of the protein.

A given amino acid may be replaced by a residue having similar physiochemical characteristics. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other conservative substitutions, e.g., involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

The invention further includes Lerk-8 polypeptides with or without associated native-pattern glycosylation. Lerk-8 expressed in yeast or mammalian expression systems (e.g., COS-7 cells) may be similar to or significantly different from a native Lerk-8 polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of Lerk-8 polypeptides in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules.

N-glycosylation sites can be modified to preclude glycosylation, allowing expression of a more homogeneous, reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. The human Lerk-8 protein of SEQ ID NO:2 comprises ___ one such triplet, at amino acids 183-185 of SEQ ID NO:2. Appropriate substitutions, additions or deletions to the nucleotide sequence encoding these triplets will result in prevention of attachment of carbohydrate residues to the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 276,846.

In another example of variants, sequences encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon renaturation. Cysteine residues corresponding to the four cysteines that are conserved among the Lerk proteins are found at positions 35, 65, 77, and 129 of SEQ ID NO:2. These four cysteines desirably remain unaltered in Lerk-8 variants.

Other variants are prepared by modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. The human Lerk-8 contains such adjacent basic residue pairs at amino acids 13-14, 63-64, 151-152, 225-226, 226-227, and 227-228 of SEQ ID NO:2. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

Naturally occurring Lerk-8 variants are also encompassed by the invention. Examples of such variants are proteins that result from alternate mRNA splicing events or from proteolytic cleavage of the Lerk-8 protein. Alternate splicing of mRNA may, for example, yield a truncated but biologically active Lerk-8 protein, such as a naturally

occurring soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the Lerk-8 protein (generally from 1-5 terminal amino acids). Thus, Lerk-8 proteins in which the N-terminal residue is any of amino acids 1 to 5 of SEQ ID NO:2, and the C-terminal residue is any of amino acids 308 to 313 of SEQ ID NO:2 are specifically provided herein. For soluble Lerk-8, the C-terminal residue may be any of amino acids 192 to 197 of SEQ ID NO:2. Lerk-8 proteins in which differences from the amino acid sequence of SEQ ID NO:2 are attributable to genetic polymorphism (allelic variation among individuals producing the protein) are also contemplated herein.

One isolated Lerk-8 cDNA included a single nucleotide substitution when compared with the cDNA described in example 1. The variant Lerk-8 DNA sequence differs from the DNA sequence presented in SEQ ID NO:1, in that the nucleotide at position 1370 of the variant is cytosine (C), rather than the guanine (G) found at that position in SEQ ID NO:1. In the amino acid sequence of this Lerk-8 protein, the residue at position 298 is leucine.

Regarding the foregoing discussion of the signal peptide and various domains of Lerk-8 protein, the skilled artisan will recognize that the above-described boundaries of such regions of the protein are approximate. The boundaries of the transmembrane region (which may be predicted by using computer programs available for that purpose) may differ from those described above. Thus, soluble Lerk-8 polypeptides in which the C-terminus of the extracellular domain differs from the residue so identified above are contemplated herein. As another illustration, cleavage of a signal peptide can occur at sites other than those predicted by computer program. Further, it is recognized that a protein preparation can comprise a mixture of protein molecules having different N-terminal amino acids, due to cleavage of the signal peptide at more than one site.

Computer analysis of the human Lerk-8 protein indicates that cleavage of the signal peptide is most likely to occur after amino acid -1 of SEQ ID NO:2. Four alternative signal peptide cleavage sites predicted by computer program are (in descending order of likelihood) located after residues 3, -5, 2, and -2 of SEQ ID NO:2. Thus, mature human Lerk-8 polypeptides in which the N-terminal amino acid is selected from the residues at positions 4, -4, 3, and -1 are provided herein, in addition to the embodiment in which the N-terminal amino acid is the residue at position 1.

Variants and derivatives of native Lerk-8 proteins may be prepared by mutation of nucleotide sequences encoding native Lerk-8 polypeptides. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid

insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to introduce a desired mutation. Methods for making such alterations include those disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985); Kunkel et al. (*Methods in Enzymol.* 154:367, 1987); and U.S. Patent Nos. 4,518,584 and 4,737,462.

Lerk-8 may be modified to create Lerk-8 derivatives by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of Lerk-8 may be prepared by linking the chemical moieties to functional groups on Lerk-8 amino acid side chains or at the N-terminus or C-terminus of a Lerk-8 polypeptide or the extracellular domain thereof. Other derivatives of Lerk-8 within the scope of this invention include covalent or aggregative conjugates of Lerk-8 polypeptides with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions.

Lerk-8 polypeptide fusions can comprise peptides added to facilitate purification and identification of Lerk-8. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the Flag® peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO:3), which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the Flag® peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the Flag® peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

Lerk-8 proteins (including fragments and variants) may be tested for the ability to bind hek or elk in any suitable assay. Biological activity of a Lerk-8 variant may be determined, for example, by assaying for the variant's ability to compete with a native Lerk-8 for binding to hek or elk (i.e. competitive binding assays).

Competitive binding assays can be performed following conventional methodology. Reagents that may be employed in competitive binding assays include radiolabeled, soluble Lerk-8 and intact hek/elk-expressing cells. For example, radiolabeled soluble native Lerk-8 can be used to compete with a soluble Lerk-8 variant for binding to cell surface-bound hek or elk. Instead of intact cells, one could substitute a soluble hek/Fc or elk/Fc fusion protein

bound to a solid phase through the interaction of Protein A or Protein G (on the solid phase) with the Fc moiety. Chromatography columns that contain Protein A and Protein G include those available from Pharmacia Biotech, Inc., Piscataway, NJ. Another type of competitive binding assay utilizes radiolabeled soluble hek or elk, such as a soluble hek/Fc or elk/Fc fusion protein, and intact cells expressing Lerk-8. In yet another alternative, a Lerk-8 may be assayed for the ability to compete with one of the other Lerk proteins (Lerks 1 through 7, described above) for binding to elk or hek. Qualitative results can be obtained by competitive autoradiographic plate binding assays, while Scatchard plots (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) may be utilized to generate quantitative results.

It is possible that the Lerk-8 of the present invention will bind to other receptors of the *eph* family (see the background section). Such binding can be analyzed using a suitable assay analogous to those described above.

Uses of Lerk-8 that flow from the ability to bind elk and hek include, but are not limited to, the following. Lerk-8 finds use as a protein purification reagent. Lerk-8 polypeptides may be attached to a solid support material and used to purify hek or elk proteins by affinity chromatography. In particular embodiments, Lerk-8 fragments or fusion proteins (e.g., Lerk-8/Fc fusions) containing the receptor-binding domain of Lerk-8 are attached to a solid support by conventional procedures. As one example, chromatography columns containing functional groups that will react with functional groups on amino acid side chains of proteins are available (Pharmacia Biotech, Inc., Piscataway, NJ). Lerk-8/Fc fusion proteins can be attached to Protein A- or Protein G-containing chromatography columns through interaction with the Fc moiety.

Lerk-8 proteins also find use in purifying or identifying cells that express hek or elk on the cell surface. The Lerk-8 (or fragment or fusion thereof) is bound to a solid phase such as a column chromatography matrix or a similar suitable substrate. For example, magnetic microspheres can be coated with Lerk-8 and held in an incubation vessel through a magnetic field. Suspensions of cell mixtures containing hek/elk-expressing cells are contacted with the solid phase having Lerk-8 thereon. Cells expressing hek or elk on the cell surface bind to the fixed Lerk-8, and unbound cells then are washed away.

Alternatively, mixtures of cells suspected of containing hek/elk$^+$ cells first can be incubated with biotinylated Lerk-8. Incubation periods are typically at least one hour in duration to ensure sufficient binding to hek/elk. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides the binding of the cell to the beads. Procedures for using avidin-coated beads are known (see Berenson, et al. *J. Cell. Biochem.*, 10D:239, 1986). Washing of unbound material and the release of the bound cells is performed using conventional methods.

The thus-purified cell population then may be used in various *in vitro* studies or *in vivo* procedures, e.g., to repopulate tissues in a mammal. To illustrate, neural cells expressing elk may be isolated by the foregoing procedure, then administered to a mammal afflicted with a neurodegenerative disorder. Hek+ cells include certain leukemia cells (identified below). Isolated leukemia cells can be used in studies of the effects of various drugs on the cells, for example.

To identify additional types of cells that express hek or elk on the cell surface, Lerk-8 can be conjugated to a detectable moiety such as a radionuclide. As one example, radiolabeling with ^{125}I can be performed by any of several standard methodologies that yield a functional ^{125}I-Lerk-8 molecule labeled to high specific activity. Other detectable moieties include enzymes that can catalyze a colorimetric or fluorometric reaction. Cells to be tested for hek/elk-expression are contacted with labeled Lerk-8. After incubation, unbound labeled Lerk-8 is removed and the presence or absence of the detectable moiety on the cells is determined.

Lerk-8 proteins also find use in measuring the biological activity of elk or hek proteins in terms of their binding affinity for Lerk-8. Lerk-8 proteins thus may be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of elk or hek protein under different conditions. To illustrate, Lerk-8 may be employed in a binding affinity study to measure the biological activity of an elk protein that has been stored at different temperatures, or produced in different cell types. Lerk-8 also may be used to determine whether biological activity is retained after modification of an elk or hek protein (e.g., chemical modification, truncation, mutation, etc.). The binding affinity of the modified elk protein for Lerk-8 is compared to that of an unmodified elk protein to detect any adverse impact of the modifications on biological activity of elk. Likewise, the biological activity of a hek protein can be assessed using Lerk-8. The biological activity of an elk or hek protein thus can be ascertained before it is used in a research study, for example.

Lerk-8 polypeptides also find use as carriers for delivering agents attached thereto to cells bearing the elk or hek cell surface receptor. Expression of hek antigen has been reported for certain leukemic cell lines, including the human T-cell leukemia cell lines designated JM and HSB-2 and the human pre-B cell leukemia cell line designated LK63 (Wicks et al., *Proc. Natl. Acad. Sci. USA*, 89:1611, 1992; Boyd et al., *J. Biol. Chem.* 267:3262, 1992. Lerk-8 proteins thus can be used to deliver diagnostic or therapeutic agents to these cells (or to other cell types found to express hek or elk on the cell surface) in *in vitro* or *in vivo* procedures.

One example of such use is to expose a hek+ leukemic cell line to a therapeutic agent/Lerk-8 conjugate to assess whether the agent exhibits cytotoxicity toward the

leukemic cells. A number of different therapeutic agents attached to Lerk-8 may be included in an assay to detect and compare the cytotoxic effect of the agents on the leukemic cells. Lerk-8/diagnostic agent conjugates may be employed to detect the presence of hek+ cells *in vitro* or *in vivo*.

Detectable (diagnostic) and therapeutic agents that may be attached to a Lerk-8 polypeptide include, but are not limited to, drugs, toxins, radionuclides, chromophores, enzymes that catalyze a colorimetric or fluorometric reaction, and the like, with the particular agent being chosen according to the intended application. Examples of drugs include those used in treating various forms of cancer, e.g., nitrogen mustards such as L-phenylalanine nitrogen mustard or cyclophosphamide, intercalating agents such as cis-diaminodichloroplatinum, antimetabolites such as 5-fluorouracil, vinca alkaloids such as vincristine, and antibiotics such as bleomycin, doxorubicin, daunorubicin, and derivatives thereof. Among the toxins are ricin, abrin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, mycotoxins such as trichothecenes, and derivatives and fragments (e.g., single chains) thereof. Radionuclides suitable for diagnostic use include, but are not limited to, ^{123}I, ^{131}I, ^{99m}Tc, ^{111}In, and ^{76}Br. Radionuclides suitable for therapeutic use include, but are not limited to, ^{131}I, ^{211}At, ^{77}Br, ^{186}Re, ^{188}Re, ^{212}Pb, ^{212}Bi, ^{109}Pd, ^{64}Cu, and ^{67}Cu.

Such agents may be attached to the Lerk-8 by any suitable conventional procedure. Lerk-8, being a protein, comprises functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the protein or agent may be derivatized to generate or attach a desired reactive functional group. The derivatization may involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to proteins (Pierce Chemical Company, Rockford, Illinois). A number of techniques for radiolabeling proteins are known. Radionuclide metals may be attached to Lerk-8 by using a suitable bifunctional chelating agent, for example.

Conjugates comprising Lerk-8 and a suitable diagnostic or therapeutic agent (preferably covalently linked) are thus prepared. The conjugates are administered or otherwise employed in an amount appropriate for the particular application.

Another use of the Lerk-8 of the present invention is as a research tool for studying the role that Lerk-8, in conjunction with elk or hek, may play in growth or differentiation of cells bearing the elk or hek receptor. The Lerk-8 polypeptides of the present invention also may be employed in *in vitro* assays for detection of elk or Lerk-8 or the interactions thereof. Likewise, Lerk-8 finds use in assays for hek or the interaction of Lerk-8 with hek. The possibility that hek plays a role in tumorigenesis has been suggested (Boyd et al., *supra*).

The Lerk-8 DNA and polypeptides of the present invention may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of, Lerk-8. Lerk-8 polypeptides may be administered to a mammal afflicted with such a disorder. Alternatively, a gene therapy approach may be taken. Disclosure herein of native Lerk-8 nucleotide sequences permits the detection of defective Lerk-8 genes, and the replacement thereof with normal Lerk-8-encoding genes. Defective genes may be detected in *in vitro* diagnostic assays, and by comparision of a native Lerk-8 nucleotide sequence disclosed herein with that of a Lerk-8 gene derived from a person suspected of harboring a defect in this gene.

As discussed above, when various rat tissues were analyzed for elk mRNA, transcripts were detected only in brain and testis (Lhotak et al., *supra*). Expression of receptors for Lerk proteins on neural tissues has led to investigation of the roles that Lerk proteins may play in development or regeneration of the nervous system. Lerk-7 has been reported to be involved in axon guidance and axon bundle formation (Winslow et al., *Neuron* 14:973-981, 1995; Drescher et al., *Cell* 82:359-370, 1995). The Lerk-8 of the present invention may be employed in studies of the effects of binding of Lerk-8 to receptors on neural tissue. The role that Lerk-8 may play in inducing or regulating processes associated with the nervous system can be investigated. Lerk-8 may be administered in vivo to regulate or promote development of the nervous system.

Certain of the above-described Lerk proteins have been reported to possess neuroprotective properties, e.g., to protect hippocampal neurons against glutamate-mediated excitotoxicity. The involvement of an excitotoxic component in a number of disorders of the neural system has been established. Responsiveness to glutamate is a normal function in the developing and mature central nervous system (CNS). In addition to its normal role in excitatory synaptic transmission and plasticity, however, glutamate can also mediate or otherwise participate in a number of CNS dysfunctional states, including but not limited to Alzheimer's disease, Huntington's Disease, Parkinsonism, stroke (ischemia), epilepsy, and AIDS-related dementia (reviewed in Meldrum and Garthwaite, *Trends Pharmacol. Sci.* 11:379, 1990; Choi, *J. Neurosci.* 10:2493, 1990; Lipton et al., *Neuron* 7:111, 1991; and Andersson et al., *Eur. J. Neurosci.* 3:66, 1991).

The Lerk-8 polypeptides provided herein find use in a method for treating disorders of neural tissue, involving contacting the neural tissue with Lerk-8. Such disorders include injury to neural tissue, or neurologic diseases, either chronic or acute. Examples of such disorders include, but are not limited to, the above-described conditions involving disfunction of the CNS. Lerk-8 may be administered to a mammal, including a human, affected with such a condition.

Certain of the Lerk proteins have been found to promote angiogenesis. Lerk-8 likewise may find use in promoting angiogenesis, which can be beneficial for wound healing, stimulating neovascularization of grafted tissues, or in treating any condition in which increased angiogenesis is desired.

Compositions comprising an effective amount of a Lerk-8 polypeptide of the present invention, in combination with other components such as a physiologically acceptable diluent, carrier, or excipient, are provided herein. Lerk-8 can be formulated according to known methods used to prepare pharmaceutically useful compositions. Lerk-8 can be combined in admixture, either as the sole active material or with other known active materials, with pharmaceutically suitable diluents (e.g., saline, Tris-HCl, acetate, and phosphate buffered solutions), preservatives (e.g., thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable formulations for pharmaceutical compositions include those described in *Remington's Pharmaceutical Sciences*, 16th ed. 1980, Mack Publishing Company, Easton, PA.

In addition, such compositions can contain Lerk-8 complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, dextran, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance of Lerk-8, and are thus chosen according to the intended application. Lerk-8 also can be conjugated to antibodies directed against tissue-specific receptors, ligands or antigens, or coupled to ligands of tissue-specific receptors. Lerk-8 expressed on the surface of a cell may find use, as well.

Such compositions may contain a Lerk-8 polypeptide in any form described herein, such as native proteins, variants, derivatives, oligomers, and biologically active fragments. In one embodiment, the composition comprises a soluble Lerk-8 polypeptide, preferably an oligomer comprising soluble Lerk-8 polypeptides.

Lerk-8 can be administered in any suitable manner, e.g., topically, parenterally, or by inhalation. The term "parenteral" includes injection, e.g., by subcutaneous, intravenous, or intramuscular routes, also including localized administration, e.g., at a site of disease or injury. Sustained release from implants is also contemplated. One skilled in the pertinent art will recognize that suitable dosages will vary, depending upon such factors as the nature of the disorder to be treated, the patient's body weight, age, and general condition, and the route of administration. Preliminary doses can be determined according to animal tests, and the scaling of dosages for human administration are performed according to art-accepted practices.

Oligomeric Forms of Lerk-8

Encompassed by the present invention are oligomers that contain Lerk-8 polypeptides. Lerk-8 oligomers may be in the form of covalently-linked or non-covalently-linked dimers, trimers, or higher oligomers.

One embodiment of the invention is directed to oligomers comprising multiple Lerk-8 polypeptides joined *via* covalent or non-covalent interactions between peptide moieties fused to the Lerk-8 polypeptides. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of Lerk-8 polypeptides attached thereto, as described in more detail below.

In particular embodiments, the oligomers comprise from two to four Lerk-8 polypeptides. The Lerk-8 moieties of the oligomer may be soluble polypeptides, as described above.

As one alternative, a Lerk-8 oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al. (*PNAS USA* 88:10535, 1991); Byrn et al. (*Nature* 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1 - 10.19.11, 1992).

One embodiment of the present invention is directed to a Lerk-8 dimer comprising two fusion proteins created by fusing Lerk-8 to the Fc region of an antibody. The Fc polypeptide preferably is fused to the C-terminus of a soluble Lerk-8. A gene fusion encoding the Lerk-8/Fc fusion protein is inserted into an appropriate expression vector. Lerk-8/Fc fusion proteins are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent Lerk-8.

Provided herein are fusion proteins comprising a Lerk-8 polypeptide fused to an Fc polypeptide derived from an antibody. DNA encoding such fusion proteins, as well as dimers containing two fusion proteins joined *via* disulfide bonds between the Fc moieties thereof, are also provided. The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included. One suitable Fc polypeptide, described in PCT application WO 93/10151, is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum et al., (*EMBO J.* 13:3992-

4001, 1994). The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

In other embodiments, Lerk-8 may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a Lerk-8 oligomer with as many as four Lerk-8 extracellular regions.

Alternatively, the oligomer is a fusion protein comprising multiple Lerk-8 polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA sequences encoding Lerk-8, using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between sequences encoding Lerk-8. In one embodiment, a fusion protein comprises from two to four soluble Lerk-8 polypeptides, separated by peptide linkers.

Another method for preparing oligomeric Lerk-8 involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al. (*FEBS Letters* 344:191, 1994) and U.S. Patent application serial no. 08/446,922, hereby incorporated by reference. Recombinant fusion proteins comprising a soluble Lerk-8 polypeptide fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric Lerk-8 that forms is recovered from the culture supernatant.

Oligomeric Lerk-8 has the property of bivalent, trivalent, etc. binding sites for elk or hek. The above-described fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

Expression Systems

Suitable host cells for expression of Lerk-8 polypeptides include prokaryotes, yeast or higher eukaryotic cells. Appropriate cloning and expression vectors for use with

bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce Lerk-8 polypeptides using RNAs derived from DNA constructs disclosed herein.

The expression vector may include DNA encoding a signal or leader peptide fused to the N-terminus of a Lerk-8 polypeptide. The signal or leader peptide co-translationally or post-translationally directs transfer of the Lerk-8 from its site of synthesis to a site inside or outside of the cell membrane or cell wall. The signal or leader peptide is cleaved from the mature Lerk-8 polypeptide. The choice of signal or leader peptide is dependent on the type of host cell that is to be employed.

Suitable prokaryotic host cells for transformation include, for example, *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various other species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. In a prokaryotic host cell, such as *E. coli*, a Lerk-8 polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant Lerk-8 polypeptide.

Lerk-8 polypeptides may be expressed in yeast host cells, preferably from the genus *Saccharomyces* (e.g., *S. cerevisiae*). Other genera of yeast, such as *Pichia* , *K. lactis* or *Kluyveromyces*, may also be employed. Yeast vectors may contain an origin of replication sequence from a 2μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene.

Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Another alternative is the glucose-repressible ADH2 promoter described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657 or in Fleer et. al., *Gene*, 107:285-195 (1991); and van den Berg et. al., *Bio/Technology*, 8:135-139 (1990). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

A suitable leader sequence (e.g. the α-factor leader of *Saccharomyces*) may be employed to direct secretion of the Lerk-8 polypeptide from yeast cells. The α-factor leader sequence is generally inserted between the promoter sequence and the structural gene sequence. See, e.g., Kurjan et al., *Cell* 30:933, 1982; Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984; U. S. Patent 4,546,082; and EP 324,274. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978. The Hinnen et al. protocol selects for Trp^+ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μg/ml adenine and 20 μg/ml uracil.

Yeast host cells transformed by vectors containing ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μg/ml adenine and 80 μg/ml uracil. Derepression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or insect host cell culture systems could also be employed to express recombinant Lerk-8 polypeptides. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651; Gluzman et al., *Cell* 23:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, the BHK (ATCC CRL 10) cell line, and the CV-1/EBNA-1 cell line (ATCC CRL 10478) derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J.* 10: 2821, 1991).

Transcriptional and translational control sequences for mammalian host cell expression vectors may be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from Polyoma virus, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment

which may also contain a viral origin of replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Examples of expression vectors for use in mammalian host cells are those constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature* 312:768, 1984 has been deposited as ATCC 39890. Other expression vectors suitable for use in mammalian host cells are pDC201 (Sims et al., *Science* 241:585, 1988), pDC302 (Mosley et al., *Cell*, 59:335, 1989), pDC406 (McMahan et al., *EMBO J.* 10:2821, 1991), HAV-EO (Dower et al., *J. Immunol.* 142:4314, 1989), and the vectors described in EP-A-0367566 and WO 91/18982. Further alternatives are vectors derived from retroviruses.

In place of the native signal sequence, a heterologous signal sequence may be added, such as the signal sequence for IL-7 described in United States Patent 4,965,195; the signal sequence for IL-2 receptor described in Cosman et al., *Nature* 312:768 (1984); the IL-4 receptor signal peptide described in EP 367,566; the type I IL-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II IL-1 receptor signal peptide described in EP 460,846.

Lerk-8 Protein Purification

Lerk-8 polypeptides of the present invention may be produced by recombinant expression systems as described above, or purified from naturally occurring cells. One process for producing Lerk-8 comprises culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes Lerk-8 under conditions sufficient to promote expression of Lerk-8. Lerk-8 is then recovered from the culture medium or cell extracts, depending upon the expression system employed and whether the Lerk-8 is secreted from the cells. In one embodiment, a human Lerk-8 protein comprises the amino acid sequence of the protein that is expressed by host cells transformed with an expression vector containing the Lerk-8 cDNA found in strain ATCC 97441.

As is known to the skilled artisan, procedures for purifying a recombinant protein will vary according to such factors as the type of host cells employed and whether or not the recombinant protein is secreted into the culture medium. Other considerations include the types of contaminants that are to be removed, which may vary according to the particular host cells employed to express the desired protein.

For example, when expression systems that secrete the recombinant protein are employed, the culture medium first may be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration matrix. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other support materials commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. In addition, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (e.g., silica gel having pendant methyl or other aliphatic groups) can be employed. Some or all of the foregoing purification steps, in various combinations, may be employed to provide a purified Lerk-8 protein.

A further alternative is affinity chromatography, employing a chromatography matrix containing hek, elk, or an antibody that binds Lerk-8. The Lerk-8 polypeptides can be recovered from an affinity column using conventional techniques, (e.g., elution in a high salt buffer), then dialyzed into a lower salt buffer for use.

Recombinant protein produced in bacterial culture can be isolated by initial disruption of the host cells, centrifugation, extraction from cell pellets if an insoluble polypeptide, or from the supernatant fluid if a soluble polypeptide, followed by one or more concentration, salting-out, ion exchange, affinity purification or size exclusion chromatography steps. Finally, RP-HPLC can be employed for final purification steps. Microbial cells can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

In yeast host cells, Lerk-8 is preferably expressed as a secreted polypeptide, to simplify purification. Recombinant polypeptides secreted from a yeast host cell fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). Urdal et al. describe two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

The desired degree of purity depends on the intended use of the protein. A relatively high degree of purity is desired when the protein is to be administered *in vivo*, for example. Advantageously, Lerk-8 polypeptides are purified such that no protein bands corresponding to other (non-Lerk-8) proteins are detectable upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). It will be recognized by one skilled in the pertinent field that multiple bands corresponding to Lerk-8 protein may be visualized by

SDS-PAGE, due to differential glycosylation, differential post-translational processing, and the like, as discussed above. Lerk-8 most preferably is purified to substantial homogeneity, as indicated by a single protein band upon analysis by SDS-PAGE. The protein band may be visualized by silver staining, Coomassie blue staining, or (if the protein is radiolabeled) by autoradiography.

Nucleic Acids and Uses Thereof

The present invention provides isolated Lerk-8 nucleic acids useful in the production of Lerk-8 polypeptides, as discussed above. Such nucleic acids include, but are not limited to, the human Lerk-8 DNA of SEQ ID NO:1, in both single-stranded and double-stranded form, as well as the RNA complement thereof. Lerk-8 DNA of the present invention includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques using the cDNA isolated in example 1, or a suitable fragment thereof, as a probe.

Particular embodiments of Lerk-8-encoding DNAs include a DNA comprising nucleotides 398 to 1420 of SEQ ID NO:1 (encoding full length human Lerk-8, including the N-terminal signal peptide) and a DNA comprising nucleotides 479 to 1420 of SEQ ID NO:1 (encoding full length mature human Lerk-8). Particular embodiments of DNA encoding a soluble human Lerk-8 are a DNA comprising nucleotides 398 to 1069 of SEQ ID NO:1 (encoding the signal peptide and extracellular domain) or comprising nucleotides 479 to 1069 of SEQ ID NO:1 (encoding the extracellular domain).

The present invention further provides fragments of Lerk-8 nucleotide sequences. Such fragments desirably comprise at least about 17 contiguous nucleotides of a Lerk-8 DNA sequence, e.g., at least 17 consecutive nucleotides of the human Lerk-8 sequence presented in SEQ ID NO:1. DNA and RNA complements of said fragments are provided herein, along with both single-stranded and double-stranded forms of the Lerk-8 DNA.

Among the uses of such Lerk-8 nucleic acid fragments is use as a probe. Such probes may be employed in cross-species hybridization procedures to isolate Lerk-8 DNA from additional mammalian species. As one example, a probe corresponding to the extracellular domain of a Lerk-8 may be employed. The probes also find use in detecting the presence of Lerk-8 nucleic acids in *in vitro* assays and in such procedures as Northern and Southern blots. Cell types expressing Lerk-8 can be identified. Such procedures are well known, and the skilled artisan can choose a probe of suitable length, depending on the particular intended application. In particular embodiments, Lerk-8 nucleic acid molecules comprise at least 30 contiguous nucleotides of the DNA sequence of SEQ ID NO:1, or the

DNA or RNA complement thereof. The probes may be labeled (e.g., with ^{32}P) by conventional techniques.

Lerk-8 nucleic acid fragments also find use as primers, e.g., in polymerase chain reactions (PCR). 5' and 3' primers corresponding to the termini of a desired Lerk-8 DNA (e.g., a DNA encoding a soluble Lerk-8) are employed in isolating and amplifying the DNA, using conventional PCR techniques.

Other useful fragments of the Lerk-8 nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target Lerk-8 mRNA (sense) or Lerk-8 DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of Lerk-8 cDNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (*Cancer Res.* 48:2659, 1988) and van der Krol et al. (*BioTechniques* 6:958, 1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block transcription or translation of the target sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of Lerk-8 proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, $CaPO_4$-mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. In a preferred procedure, an antisense or sense oligonucleotide is

inserted into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, those derived from the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see WO 90/13641).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

Antibodies

Antibodies that are immunoreactive with Lerk-8 polypeptides are provided herein. Such antibodies specifically bind Lerk-8, in that the antibodies bind to Lerk-8 *via* the antigen-binding sites of the antibody (as opposed to non-specific binding).

Polyclonal and monoclonal antibodies may be prepared by conventional techniques. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988). Production of monoclonal antibodies directed against Lerk-8 is further illustrated in example 3.

Antigen-binding fragments of such antibodies, which may be produced by conventional techniques, are also encompassed by the present invention. Examples of such fragments include, but are not limited to, Fab, F(ab'), and $F(ab')_2$ fragments. Antibody fragments and derivatives produced by genetic engineering techniques are also provided.

The monoclonal antibodies of the present invention include chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen

binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature 332*:323, 1988), Liu et al. (*PNAS 84*:3439, 1987), Larrick et al. (*Bio/Technology 7*:934, 1989), and Winter and Harris (*TIPS 14*:139, May, 1993).

Among the uses of the antibodies are use in assays to detect the presence of Lerk-8 polypeptides, either *in vitro* or *in vivo*. The antibodies also may be employed in purifying Lerk-8 proteins by immunoaffinity chromatography.

Those antibodies that additionally can block binding of Lerk-8 to receptors (e.g., elk or hek) may be used to inhibit a biological activity mediated by the binding of Lerk-8 to the receptors. Such an antibody may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit a Lerk-8-mediated biological activity. Disorders mediated or exacerbated (directly or indirectly) by the binding of Lerk-8 to cell surface receptors are thus treated.

Pharmaceutical compositions comprising an antibody that is directed against Lerk-8, and a suitable, diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described above for compositions containing Lerk-8 proteins.

Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to an antibody directed against Lerk-8. Examples of such agents are presented above. The conjugates find use in *in vitro* or *in vivo* procedures.

The following examples are provided to further illustrate particular embodiments of the invention, and are not to be construed as limiting the scope of the present invention.

EXAMPLE 1: Cloning of Human Lerk-8 cDNA

A cDNA encoding a human Lerk-8 of the present invention was isolated by the following procedure. A search of the GenBank sequence databank (tfasta), using amino acid sequences of Lerk-2 and Lerk-5 as search terms, identified an EST (accession no. H10006) exhibiting significant homology. Using the reading frames of Lerks 2 and 5 as guides (to adjust for frame shifts and stop codons that would result from the insertions and deletions in the EST, compared to the Lerk-2 and Lerk-5 sequences), a translate of the EST was elucidated. Alignment of this translate of the EST with Lerk-2 and Lerk-5 amino acid sequences revealed sequence identity of about 50% with both Lerk-2 and Lerk-5, in the overlapping regions. The second, third, and fourth cysteines that are conserved in Lerks 1-7 were identified in the EST translate.

Oligonucleotides based on the EST were synthesized for use as 5' and 3' primers in a polymerase chain reaction (PCR). The primers defined the termini of a 110bp internal

fragment of the EST. DNA from human cDNA libraries in a phage λ vector was used as the template in the PCR. DNA fragments of the expected size (110bp) were amplified from three of the cDNA libraries, which were derived from fetal brain, dermal fibroblast, and pancreatic tumor.

The same two oligonucleotides used as primers in the PCR were end labeled with ^{32}P for use as probes. The human dermal fibroblast cDNA library was screened with the probes by allowing hybridization at 63°C, followed by washing at 63°C in 1x SSC. One hybridizing clone, designated λ1 was isolated. The coding region of this clone corresponds to nucleotides 500 to 1420 of SEQ ID NO:1, which encode amino acids 8 through 313 of SEQ ID NO:2.

A fragment of this clone was amplified by PCR, labeled, and used as a probe in screening the human fetal brain cDNA library (hybridization at 63°C, followed by washing at 63°C in 1x SSC). Three hybridizing clones were isolated, and the DNA sequences were determined. One clone, designated λ2, included a full length coding region.

The nucleotide sequence of the human Lerk-8 cDNA of clone λ2, and the amino acid sequence encoded thereby, are presented in SEQ ID NO:1 and SEQ ID NO:2, respectively. The human Lerk-8 protein of SEQ ID NO:2 comprises an N-terminal signal peptide (amino acids -27 to -1), an extracellular domain (amino acids 1 to 197), a transmembrane region (amino acids 198 to 224), and a cytoplasmic domain (amino acids 225 to 313).

Samples of a cell lysate containing a recombinant phage vector (λgt10 containing the human Lerk-8 cDNA of clone λ2 inserted into the EcoRI restriction site of the vector) were deposited with the American Type Culture Collection, Rockville, Maryland. The samples were deposited on February 14, 1996, under the terms of the Budapest Treaty, and were assigned accession number ATCC 97441.

EXAMPLE 2: Binding Study

The binding of Lerk-8 to elk or hek can be assessed in any conventional binding assay. One suitable procedure is as follows.

A DNA and encoded amino acid sequence for rat elk cDNA is disclosed in Lhotak et al. (*Mol. Cell. Biol.* 11:2496, 1991), hereby incorporated by reference. The rat elk protein has a 538 amino acid extracellular domain, a 25 amino acid transmembrane domain, and a 419 amino acid cytoplasmic domain.

A DNA and encoded amino acid sequence for human hek cDNA is presented in Wicks et al. (*Proc. Natl. Acad. Sci. USA*, 89:1611, 1992), incorporated herein by reference. This hek protein comprises (from N- to C-terminus) a 521 amino acid

extracellular domain, a 24 amino acid transmembrane domain, and a 418 amino acid cytoplasmic domain.

Recombinant soluble elk/Fc and hek/Fc fusion proteins are prepared by any suitable procedure, e.g., as described in PCT application WO 96/01839, hereby incorporated by reference. The elk/Fc and hek/Fc fusion proteins are purified by affinity chromatography, using a protein A sepharose column.

Cells expressing recombinant Lerk-8 on the cell surface are prepared. Lerk-8 DNA may be amplified by PCR. The primers employed in the PCR are selected to define the termini of the coding region of the Lerk-8 DNA, and also add an Xho I restriction site at the 5' end and a Not I site at the 3' end of the amplified DNA.

The PCR reaction products are digested with Xho I and Not I and inserted into an expression vector cleaved with Sal I (which is compatible with Xho I) and Not I. The expression vector, designated pDC410, is a mammalian expression vector that also replicates in *E. coli*, and is similar to pDC406 (McMahan et al., *EMBO J. 10*:2821, 1991). The pDC410 multiple cloning site (mcs) differs from that of pDC406 in that it contains additional restriction sites and three stop codons (one in each reading frame). A T7 polymerase promoter downstream of the mcs facilitates sequencing of DNA inserted into the mcs. In addition, the EBV origin of replication is replaced by DNA encoding the SV40 large T antigen (driven from an SV40 promoter) in pDC410.

CV1-EBNA-1 cells in 10 cm^2 dishes are transfected with the recombinant expression vector containing Lerk-8 DNA. The CV-1/EBNA-1 cell line (ATCC CRL 10478) constitutively expresses EBV nuclear antigen-1 driven from the CMV immediate-early enhancer/promoter. CV1-EBNA-1 was derived from the African Green Monkey kidney cell line CV-1 (ATCC CCL 70), as described by McMahan et al. (*EMBO J.* 10:2821, 1991).

The transfected cells are cultured for 24 hours, and the cells in each dish then are split into a 24-well plate. After culturing an additional 48 hours, the transfected cells (about 4×10^4 cells/well) are washed with BM-NFDM, which is binding medium (RPMI 1640 containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, 20 mM Hepes pH 7.2) to which 50 mg/ml nonfat dry milk has been added. The cells then are incubated for 1 hour at 37°C with various concentrations of the above-described elk/Fc fusion protein or hek/Fc fusion protein. Cells then are washed and incubated with a constant saturating concentration of a ^{125}I-mouse anti-human IgG in binding medium, with gentle agitation for 1 hour at 37°C. After extensive washing, cells are released *via* trypsinization.

The mouse anti-human IgG employed above is directed against the Fc region of human IgG and can be obtained from Jackson Immunoresearch Laboratories, Inc., West Grove, PA. The antibody is radioiodinated using the standard chloramine-T method. The

antibody will bind to the Fc portion of any elk/Fc or hek/Fc fusion protein that has bound to the cells. In all assays, non-specific binding of ^{125}I-antibody is assayed in the absence of elk/Fc (or hek/Fc), as well as in the presence of elk/Fc (or hek/Fc) and a 200-fold molar excess of unlabeled mouse anti-human IgG antibody.

Cell-bound ^{125}I-antibody is quantified on a Packard Autogamma counter. Affinity calculations (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) are generated on RS/1 (BBN Software, Boston, MA) run on a Microvax computer.

EXAMPLE 3: Monoclonal Antibodies That Bind Lerk-8

This example illustrates a method for preparing monoclonal antibodies that bind Lerk-8. Suitable immunogens that may be employed in generating such antibodies include, but are not limited to, purified Lerk-8 protein or an immunogenic fragment thereof such as the extracellular domain, or fusion proteins containing Lerk-8 (e.g., a soluble Lerk-8/Fc fusion protein).

Purified Lerk-8 can be used to generate monoclonal antibodies immunoreactive therewith, using conventional techniques such as those described in U.S. Patent 4,411,993. Briefly, mice are immunized with Lerk-8 immunogen emulsified in complete Freund's adjuvant, and injected in amounts ranging from 10-100 µg subcutaneously or intraperitoneally. Ten to twelve days later, the immunized animals are boosted with additional Lerk-8 emulsified in incomplete Freund's adjuvant. Mice are periodically boosted thereafter on a weekly to bi-weekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision to test for Lerk-8 antibodies by dot blot assay, ELISA (Enzyme-Linked Immunosorbent Assay) or inhibition of hek or elk binding.

Following detection of an appropriate antibody titer, positive animals are provided one last intravenous injection of Lerk-8 in saline. Three to four days later, the animals are sacrificed, spleen cells harvested, and spleen cells are fused to a murine myeloma cell line, e.g., NS1 or preferably P3x63Ag8.653 (ATCC CRL 1580). Fusions generate hybridoma cells, which are plated in multiple microtiter plates in a HAT (hypoxanthine, aminopterin and thymidine) selective medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells are screened by ELISA for reactivity against purified Lerk-8 by adaptations of the techniques disclosed in Engvall et al., *Immunochem.* 8:871, 1971 and in U.S. Patent 4,703,004. A preferred screening technique is the antibody capture technique described in Beckmann et al., (*J. Immunol.* 144:4212, 1990) Positive hybridoma cells can be injected intraperitoneally into syngeneic BALB/c mice to produce ascites containing high concentrations of anti-Lerk-8 monoclonal antibodies. Alternatively,

hybridoma cells can be grown *in vitro* in flasks or roller bottles by various techniques. Monoclonal antibodies produced in mouse ascites can be purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to Protein A or Protein G can also be used, as can affinity chromatography based upon binding to Lerk-8.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Cerretti, Douglas P.

(ii) TITLE OF INVENTION: Cytokine Designated Lerk-8

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Kathryn A. Anderson, Immunex Corporation
(B) STREET: 51 University Street
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98101

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Power Macintosh
(C) OPERATING SYSTEM: Apple Operating System 7.5.3
(D) SOFTWARE: Microsoft Word for Power Macintosh 6.0.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: --to be assigned--
(B) FILING DATE: 19-MAR-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Anderson, Kathryn A.
(B) REGISTRATION NUMBER: 32,172
(C) REFERENCE/DOCKET NUMBER: 2839-WO

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (206) 587-0430
(B) TELEFAX: (206) 233-0644
(C) TELEX: 756822

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1708 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: huLerk8

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 398..1420

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 479..1417

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 398..478

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AATTCCGGCC CTTAGCCCGC TGCCCTCAAT CCCAGCGAGG CTGGGGCTCC GGCTCGGCGC    60

CCCCTTCCTC GCTCCCTGGT CCGGCGCCCC ATGCCGCCCC CGCCCGGTCC CCGGCTCCCC   120

CAGTCCCCCA CTTAGGCGGG CTCACAGATC CCGGGGTGCT GGCGCGTGGG CCGGGGGCGC   180

GTAGGGCGCC TGCAGACGGC CCCTGGAAGG GCTCTGGTGG GGCTGAGCGC TCTGCCGCGG   240

GGGCGCGGGC ACAGCAGGAA GCAGGTCCGC GTGGGCGCTG GGGGCATCAG CTACCGGGGT   300

GGTCCGGGCT GAAGAGCCAG GCAGCCAAGG CAGCCACCCC GGGGGGTGGG CGACTTTGGG   360

GGAGTTGGTG CCCCGCCCCC CAGGCCTTGG CGGGGTC ATG GGG CCC CCC CAT TCT     415
                                          Met Gly Pro Pro His Ser
                                          -27      -25

GGG CCG GGG GGC GTG CGA GTC GGG GCC CTG CTG CTG CTG GGG GTT TTG      463
Gly Pro Gly Gly Val Arg Val Gly Ala Leu Leu Leu Leu Gly Val Leu
    -20                 -15                 -10

GGG CTG GTG TCT GGG CTC AGC CTG GAG CCT GTC TAC TGG AAC TCG GCG      511
Gly Leu Val Ser Gly Leu Ser Leu Glu Pro Val Tyr Trp Asn Ser Ala
 -5                  1                   5                  10

AAT AAG AGG TTC CAG GCA GAG GGT GGT TAT GTG CTG TAC CCT CAG ATC      559
Asn Lys Arg Phe Gln Ala Glu Gly Gly Tyr Val Leu Tyr Pro Gln Ile
             15                  20                  25

GGG GAC CGG CTA GAC CTG CTC TGC CCC CGG GCC CGG CCT CCT GGC CCT      607
Gly Asp Arg Leu Asp Leu Leu Cys Pro Arg Ala Arg Pro Pro Gly Pro
         30                  35                  40

CAC TCC TCT CCT AAT TAT GAG TTC TAC AAG CTG TAC CTG GTA GGG GGT      655
His Ser Ser Pro Asn Tyr Glu Phe Tyr Lys Leu Tyr Leu Val Gly Gly
     45                  50                  55

GCT CAG GGC CGG CGC TGT GAG GCA CCC CCT GCC CCA AAC CTC CTT CTC      703
Ala Gln Gly Arg Arg Cys Glu Ala Pro Pro Ala Pro Asn Leu Leu Leu
 60                  65                  70                  75

ACT TGT GAT CGC CCA GAC CTG GAT CTC CGC TTC ACC ATC AAG TTC CAG      751
Thr Cys Asp Arg Pro Asp Leu Asp Leu Arg Phe Thr Ile Lys Phe Gln
                 80                  85                  90
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GAG TAT AGC CCT AAT CTC TGG GGC CAC GAG TTC CGC TCG CAC CAC GAT      799
Glu Tyr Ser Pro Asn Leu Trp Gly His Glu Phe Arg Ser His His Asp
            95                  100                 105

TAC TAC ATC ATT GCC ACA TCG GAT GGG ACC CGG GAG GGC CTG GAG AGC      847
Tyr Tyr Ile Ile Ala Thr Ser Asp Gly Thr Arg Glu Gly Leu Glu Ser
        110                 115                 120

CTG CAG GGA GGT GTG TGC CTA ACC AGA GGC ATG AAG GTG CTT CTC CGA      895
Leu Gln Gly Gly Val Cys Leu Thr Arg Gly Met Lys Val Leu Leu Arg
    125                 130                 135

GTG GGA CAA AGT CCC CGA GGA GGG GCT GTC CCC CGA AAA CCT GTG TCT      943
Val Gly Gln Ser Pro Arg Gly Gly Ala Val Pro Arg Lys Pro Val Ser
140                 145                 150                 155

GAA ATG CCC ATG GAA AGA GAC CGA GGG GCA GCC CAC AGC CTG GAG CCT      991
Glu Met Pro Met Glu Arg Asp Arg Gly Ala Ala His Ser Leu Glu Pro
                160                 165                 170

GGG AAG GAG AAC CTG CCA GGT GAC CCC ACC AGC AAT GCA ACC TCC CGG     1039
Gly Lys Glu Asn Leu Pro Gly Asp Pro Thr Ser Asn Ala Thr Ser Arg
            175                 180                 185

GGT GCT GAA GGC CCC CTG CCC CCT CCC AGC ATG CCT GCA GTG GCT GGG     1087
Gly Ala Glu Gly Pro Leu Pro Pro Pro Ser Met Pro Ala Val Ala Gly
        190                 195                 200

GCA GCA GGG GGG CTG GCG CTG CTC TTG CTG GGC GTG GCA GGG GCT GGG     1135
Ala Ala Gly Gly Leu Ala Leu Leu Leu Leu Gly Val Ala Gly Ala Gly
    205                 210                 215

GGT GCC ATG TGT TGG CGG AGA CGG CGG GCC AAG CCT TCG GAG AGT CGC     1183
Gly Ala Met Cys Trp Arg Arg Arg Arg Ala Lys Pro Ser Glu Ser Arg
220                 225                 230                 235

CAC CCT GGT CCT GGC TCC TTC GGG AGG GGA GGG TCT CTG GGC CTG GGG     1231
His Pro Gly Pro Gly Ser Phe Gly Arg Gly Gly Ser Leu Gly Leu Gly
                240                 245                 250

GGT GGA GGT GGG ATG GGA CCT CGG GAG GCT GAG CCT GGG GAG CTA GGG     1279
Gly Gly Gly Gly Met Gly Pro Arg Glu Ala Glu Pro Gly Glu Leu Gly
            255                 260                 265

ATA GCT CTG CGG GGT GGC GGG GCT GCA GAT CCC CCC TTC TGC CCC CAC     1327
Ile Ala Leu Arg Gly Gly Gly Ala Ala Asp Pro Pro Phe Cys Pro His
        270                 275                 280

TAT GAG AAG GTG AGT GGT GAC TAT GGG CAT CCT GTG TAT ATC GTG CAG     1375
Tyr Glu Lys Val Ser Gly Asp Tyr Gly His Pro Val Tyr Ile Val Gln
    285                 290                 295

GAT GGG CCC CCC CAG AGC CCT CCA AAC ATC TAC TAC AAG GTA TGA         1420
Asp Gly Pro Pro Gln Ser Pro Pro Asn Ile Tyr Tyr Lys Val  *
300                 305                 310

GGGCTCCTCT CACGTGGCTA TCCTGAATCC AGCCCTTCTT GGGGTGCTCC TCCAGTTTAA  1480

TTCCTGGTTT GAGGGACACC TCTAACATCT CGGCCCCCTG TGCCCCCCCA GCCCCTTCAC  1540
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TCCTCCCGGC TGCTGTCCTC GTCTCCACTT TTAGGATTCC TTAGGATTCC CACTGCCCCA 1600

CTTCCTGCCC TCCCGTTTGG CCATGGGTGC CCCCCTCTGT CTCAGTGTCC CTGGATCCTT 1660

TTTCCTTGGG GAGGGGCACA GGCTCAGCCT CCTCTCTGAC CATGCCGG 1708

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 341 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Gly Pro Pro His Ser Gly Pro Gly Gly Val Arg Val Gly Ala Leu
-27      -25                 -20                 -15

Leu Leu Leu Gly Val Leu Gly Leu Val Ser Gly Leu Ser Leu Glu Pro
    -10                  -5                   1                   5

Val Tyr Trp Asn Ser Ala Asn Lys Arg Phe Gln Ala Glu Gly Gly Tyr
                10                  15                  20

Val Leu Tyr Pro Gln Ile Gly Asp Arg Leu Asp Leu Leu Cys Pro Arg
            25                  30                  35

Ala Arg Pro Pro Gly Pro His Ser Ser Pro Asn Tyr Glu Phe Tyr Lys
        40                  45                  50

Leu Tyr Leu Val Gly Gly Ala Gln Gly Arg Arg Cys Glu Ala Pro Pro
    55                  60                  65

Ala Pro Asn Leu Leu Leu Thr Cys Asp Arg Pro Asp Leu Asp Leu Arg
70                  75                  80                  85

Phe Thr Ile Lys Phe Gln Glu Tyr Ser Pro Asn Leu Trp Gly His Glu
                90                  95                  100

Phe Arg Ser His His Asp Tyr Tyr Ile Ile Ala Thr Ser Asp Gly Thr
            105                 110                 115

Arg Glu Gly Leu Glu Ser Leu Gln Gly Gly Val Cys Leu Thr Arg Gly
        120                 125                 130

Met Lys Val Leu Leu Arg Val Gly Gln Ser Pro Arg Gly Gly Ala Val
    135                 140                 145

Pro Arg Lys Pro Val Ser Glu Met Pro Met Glu Arg Asp Arg Gly Ala
150                 155                 160                 165

Ala His Ser Leu Glu Pro Gly Lys Glu Asn Leu Pro Gly Asp Pro Thr
                170                 175                 180

Ser Asn Ala Thr Ser Arg Gly Ala Glu Gly Pro Leu Pro Pro Pro Ser
            185                 190                 195
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Met Pro Ala Val Ala Gly Ala Ala Gly Gly Leu Ala Leu Leu Leu Leu
        200                 205                 210

Gly Val Ala Gly Ala Gly Gly Ala Met Cys Trp Arg Arg Arg Arg Ala
    215                 220                 225

Lys Pro Ser Glu Ser Arg His Pro Gly Pro Gly Ser Phe Gly Arg Gly
230                 235                 240                 245

Gly Ser Leu Gly Leu Gly Gly Gly Gly Gly Met Gly Pro Arg Glu Ala
                250                 255                 260

Glu Pro Gly Glu Leu Gly Ile Ala Leu Arg Gly Gly Gly Ala Ala Asp
            265                 270                 275

Pro Pro Phe Cys Pro His Tyr Glu Lys Val Ser Gly Asp Tyr Gly His
        280                 285                 290

Pro Val Tyr Ile Val Gln Asp Gly Pro Pro Gln Ser Pro Pro Asn Ile
    295                 300                 305

Tyr Tyr Lys Val  *
310
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: FLAG peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Asp Tyr Lys Asp Asp Asp Asp Lys
  1
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CLAIMS

What is claimed is:

1. An isolated DNA encoding a Lerk-8 polypeptide that binds hek or elk, wherein said Lerk-8 polypeptide comprises an amino acid sequence that is at least 80% identical to a sequence selected from the group consisting of residues -27 to 313 of SEQ ID NO:2 and residues 1 to 313 of SEQ ID NO:2.

2. A DNA of claim 1, wherein said Lerk-8 polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of residues -27 to 313 of SEQ ID NO:2 and residues 1 to 313 of SEQ ID NO:2.

3. A DNA of claim 2, wherein said Lerk-8 polypeptide comprises an amino acid sequence selected from the group consisting of residues -27 to 313 of SEQ ID NO:2 and residues 1 to 313 of SEQ ID NO:2.

4. A DNA of claim 1 or 2, wherein said Lerk-8 polypeptide is naturally occurring.

5. An isolated DNA encoding a mature human Lerk-8 polypeptide that binds hek or elk, wherein said Lerk-8 polypeptide is characterized by:
a) a calculated molecular weight of about 33 kilodaltons;
b) an isoelectric point (pI) of about 8.46; and
c) an N-terminal amino acid sequence Leu-Ser-Leu-Glu-Pro-Val-Tyr-Trp-Asn-Ser-Ala-Asn- (amino acids 1-12 of SEQ ID NO:2).

6. An isolated DNA encoding a soluble Lerk-8 polypeptide that binds hek or elk, wherein said Lerk-8 polypeptide comprises an amino acid sequence that is at least 80% identical to a sequence selected from the group consisting of residues -27 to x of SEQ ID NO:2 and residues 1 to x of SEQ ID NO:2, wherein x represents an integer from 142 to 197, inclusive.

7. A DNA of claim 6, wherein said soluble Lerk-8 polypeptide comprises an amino acid sequence selected from the group consisting of residues -27 to x of SEQ ID NO:2 and residues 1 to x of SEQ ID NO:2, wherein x represents an integer from 142 to 197, inclusive.

8. An isolated DNA encoding a Lerk-8 polypeptide selected from the group consisting of:
a) the human Lerk-8 polypeptide of SEQ ID NO:2; and
b) a fragment of the polypeptide of (a), wherein said fragment is capable of binding elk or hek.

9. A DNA of claim 8, wherein said fragment is a soluble fragment.

10. An expression vector comprising a DNA of any of claims 1 to 9.

11. A host cell transformed with an expression vector of claim 10.

12. A process for producing a Lerk-8 polypeptide, comprising culturing a host cell of claim 11 under conditions that promote expression of the Lerk-8 polypeptide, and recovering the Lerk-8 polypeptide.

13. A purified Lerk-8 polypeptide, wherein said polypeptide is encoded by a DNA according to any of claims 1 to 9.

14. A purified Lerk-8 polypeptide comprising an amino acid sequence that is at least 80% identical to the sequence of residues 1 to 313 of SEQ ID NO:2.

15. A Lerk-8 polypeptide of claim 14, comprising an amino acid sequence that is at least 90% identical to the sequence of residues 1 to 313 of SEQ ID NO:2.

16. A Lerk-8 polypeptide of claim 15, comprising the amino acid sequence of residues 1 to 313 of SEQ ID NO:2.

17. A Lerk-8 polypeptide of claim 15, wherein said Lerk-8 comprises amino acids 1 through 297 and 299 through 313 of SEQ ID NO:2, wherein the residue at position 298 is leucine.

18. A purified human Lerk-8 protein that binds hek or elk, wherein a mature form of said protein is characterized by:
a) a calculated molecular weight of about 33 kilodaltons;
b) an isoelectric point (pI) of about 8.46; and

c) an N-terminal amino acid sequence Leu-Ser-Leu-Glu-Pro-Val-Tyr-Trp-Asn-Ser-Ala-Asn- (amino acids 1-12 of SEQ ID NO:2).

19. A purified soluble Lerk-8 polypeptide that binds hek or elk, wherein said Lerk-8 polypeptide comprises an amino acid sequence that is at least 80% identical to the sequence of residues 1 to x of SEQ ID NO:2, wherein x represents an integer from 142 to 197, inclusive.

20. A soluble Lerk-8 polypeptide of claim 19, wherein said Lerk-8 comprises the sequence of residues 1 to x of SEQ ID NO:2, wherein x represents an integer from 142 to 197, inclusive.

21. A purified Lerk-8 polypeptide selected from the group consisting of:
a) the human Lerk-8 polypeptide of SEQ ID NO:2; and
b) a fragment of the polypeptide of (a), wherein said fragment is capable of binding elk or hek.

22. A Lerk-8 polypeptide of claim 21, wherein said fragment is a soluble fragment.

23. An oligomer comprising from two to four Lerk-8 polypeptides according to any of claims 13 to 22.

24. An oligomer of claim 23, wherein said oligomer is a dimer comprising two soluble Lerk-8/Fc fusion proteins.

25. A pharmaceutical composition comprising a Lerk-8 polypeptide or oligomer of any of claims 13 to 24, and a suitable diluent, excipient, or carrier.

26. An antibody that is directed against a Lerk-8 polypeptide of any of claims 13 to 22.

27. An antibody according to claim 26, wherein the antibody is a monoclonal antibody.